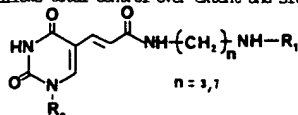


EXHIBIT 44

3666

CHEMICAL SYNTHESIS OF MODIFIED OLIGONUCLEOTIDES AND THEIR UTILITY AS NON-RADIOACTIVE HYBRIDIZATION PROBES. Jerry L. Ruth* and Robert N. Bryan*, (SPON: N. KAPLAN), Molecular Biosystems, Inc., San Diego, CA 92121

Several deoxyuridine analogs with a primary amine "linker arm" of eight or twelve atoms in length attached at C-5 (1) have been efficiently prepared. These linker arm nucleosides ("LANs") are appropriately blocked and chemically incorporated into oligonucleotides in good yields. This method allows total control over extent and site of modification in the oligomer.



Using a modified phosphochloridite method, three 22mers complementary to known sequences of herpes simplex virus (HSV) were constructed to be specific for either HSV-1, HSV-2, or both (generic). The purified oligomers were kinased and hybridized to HSV target DNA. Targets included an HSV-containing plasmid, lab strains of HSV, and clinical samples of both types 1 and 2. Results clearly demonstrated the desired selectivity. Oligonucleotides containing one or more LANs were selectively modified by attachment of fluorescein (2b) or biotin (2c). Biotinylated probes (2c) kinased and hybridized normally. Non-radioactive detection of 2c was accomplished by dye formation using an avidin-dehydrogenase complex. Results indicate such modified oligonucleotides can: 1) be made very efficiently by chemical methods in large amounts; 2) detect target DNA cleanly and specifically; and 3) be detected by non-radioactive methods.

- 1: R₁ = H
- R₂ = deoxyribose
- 2: R₂ = oligonucleotide
- a) R₁ = H
- b) R₁ = fluorescein
- c) R₁ = caproylbiotin

3668

LAMBDA PHAGE Cro REPRESSOR-DNA INTERACTIONS: FLUOROURACIL ANALOGUES OF O_g3. William T. Metzler*, Elizabeth Tecza*, Kim T. Arndt* and Ponzy Lu. Department of Chemistry, Univ. of Pennsylvania, Philadelphia, PA 19104

We have synthesized 15 base pair analogues of the center of the lambda O_g3 DNA sequence which contain deoxyfluorouracil replacements at specific thymine locations, 5 and 10, in the sequence below [indicated by * using the numbering system of Kawashima et al. (1977), Biochemistry 16, 4209-4216]:

```

2   5   10  15
ATCACC GCAAGGGAT
TAGTGG CGTTCCCTA
  *      *

```

Using quenching of the fluorescence at the tyrosines of the cro repressor molecule, we were able to demonstrate that the fluorouracil analogue of O_g3 binds to the protein as well as a 15 base pair non fluorine containing O_g3 sequence.

By observing the Fluorine-19 NMR signals in the presence and absence of the cro repressor we are able to monitor the formation of the specific cro-O_g3 complex. (Supported by grants from the NIH.)

3670

NEW METHOD FOR SEQUENCE ANALYSIS OF OLIGODEOXYRIBONUCLEOTIDES. D. M. Black* and P. T. Gilham. Purdue Univ., West Lafayette, IN 47907.

A simple sequencing procedure employs a two-dimensional separation on a single thin layer chromatographic sheet and consists of, in the first dimension, the fractionation by chain length of a nested set of fragments derived from the oligodeoxyribonucleotide by partial exonuclease action and labelled at their non-common ends, followed by *in situ* enzymatic degradation of the members of the set and separation of the resulting labelled mononucleotides in the second dimension. Spleen phosphodiesterase and alkaline phosphatase are used to produce the nested set whose members have a common 3' terminus, and these are radioactively labelled at their non-common ends with polynucleotide kinase. The fragments are separated on a polyethyleneimine-cellulose thin layer sheet, using a modification of the solvent system developed by Randerath et al. [Nucleic Acids Res. 1, 1121 (1974)]. The oligomers are then digested *in situ* to 5' mononucleotides using nuclease Bal 31, nuclease S1, or snake venom phosphodiesterase, and the labelled monomer formed at each position is identified by chromatography in the second dimension with 0.2 M NaOAc adjusted to pH 4.2 with AcOH. This approach to sequence analysis has an added advantage in permitting assignment of the identity and location of any modified or unusual base within the oligonucleotide. (Supported by CA 30234)

3667

SYNTHESIS, THERMODYNAMICS AND DRUG INTERACTIONS OF MODEL OCTANUCLEOTIDES. I. Lassalle*, H.L. Weith and S.R. Byrn*. Purdue University, West Lafayette, Indiana 47907.

Two pairs of complementary octanucleotides were chemically synthesized using the modified phosphotriester method. Their sequences are: TGACGTGA - TCACGTCA and TGAGGTGA - TCACCTCA. Optical studies were performed on the single strands in order to accurately determine their molar absorptivity coefficient (c) and their % hyperchromicity. The thermodynamics of helix formation were determined from melting curve data. In addition, the binding of the acridine AMSA to both the single strands as well as the duplexes was investigated. This data provides insight into the parameters controlling helix formation and drug binding.

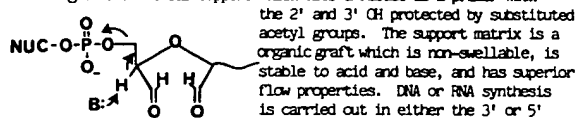
Sponsored in part by grants CA30234 and GM29175.

FILE COPY

3669

A NOVEL UNIVERSAL SUPPORT FOR DNA & RNA SYNTHESIS. L. J. Arnold, Jr.*, R. Lohmann*, and J. L. Ruth* (SPON: N. O. Kaplan), Molecular Biosystems, Inc., San Diego, CA 92121

Current methods of DNA and RNA synthesis have been greatly facilitated by the use of solid supports. Typically such supports have either a silica or organic matrix to which is attached the first nucleoside through a 3' ester linkage. Since the nucleoside can be any of four ribo- or deoxyribonucleosides, eight such supports are necessary to carry out RNA and DNA synthesis. We have designed a universal support which uses a ribose as a primer with



the 2' and 3' OH protected by substituted acetyl groups. The support matrix is an organic graft which is non-swellable, is stable to acid and base, and has superior flow properties. DNA or RNA synthesis is carried out in either the 3' or 5' direction. Once synthesis is complete, the ribose acetyl groups are removed with mild base followed by the selective oxidation of the cis diol by periodate and a β -elimination of the formed oligomer. This support has many advantages which include: 1) It is equally useful for RNA or DNA synthesis; 2) Chain elongation may go in either the 3' or 5' direction; 3) The synthesized oligomer may be base and phosphate deblocked before or after removal of the oligomer from the support; 4) The product oligomers are of higher purity since blocking groups can be removed before removal of the oligomer; and 5) After removal of protecting groups the oligomer may be left attached which permits its use as an affinity hybridization support. The functional properties of this support have been confirmed using ¹⁴C-thymidine labels. Under deblocking conditions, less than 6% of the oligomer is removed. During selective cleavage greater than 80% of the oligomer is eliminated. Using this support we have synthesized a range of 14-20 mers.

3671

NON-RADIOACTIVE BIOTIN-DEPENDENT HYBRIDIZATION/DETECTION USING UNLABELED PROBE DNA. BY: CHRISTINE L. BRAKEL*, KATHERINE MARKARIAN, AND DEAN L. ENGLEHARDT. ENZO BIOCHEM, INC. NEW YORK, NEW YORK 10013.

We have developed a method for the non-radioactive detection of DNA probes that do not contain modified nucleotides. In this system, the probe DNA is 3' terminally labeled with TTP or dATP in the presence of terminal transferase to result in a probe which contains single stranded homopolymeric termini. After this probe has been hybridized to target DNA, the hybridized molecules are complexed with a complementary homopolymer containing biotinylated nucleotides. Hybridization is visualized using a colorimetric detection system for biotin composed of streptavidin (a biotin-binding protein) and a biotinylated enzyme (either acid phosphatase or horseradish peroxidase). Addition of the appropriate substrate yields a colored precipitate. The detection sensitivity of this system was found to be greater than the sensitivity obtained using directly biotinylated probes, prepared either by nick translation or terminal labeling. This new method can be used for the detection of specific sequences on dot blots and Southern transfers.

American Society of Biological Chemists

5th Annual Meeting

and

The American Association of Immunologists

8th Annual Meeting

St. Louis, Missouri

June 3-7, 1984

ABSTRACTS OF PAPERS

2477-3780

INDEXES

Publications Committee

F. STEPHEN VOGEL, *Chairman*
JOHN S. COOK
RICHARD M. FORBES
FRANKLIN P. INMAN
HENRY KAMIN
RICHARD MCINTOSH
JOSEPH J. McPHILLIPS
ROBERT W. KRAUSS, *ex officio*
HAROLD F. HARDMAN, *ex officio*

Editorial Board

L. ROSS HACKLER, *Chairman*
MARK L. ENTMAN
DONALD M. MARCUS
EMANUEL RUBIN
ALAN N. SCHECHTER
ROBERT E. STITZEL

Executive Editor

KARL F. HEUMANN
301-530-7100

Assistant Executive Editor

JUDITH B. GANDY
301-530-7119

Copy Editor

KENDALL SITES
301-530-7108

VOLUME 43, NUMBER 7 • MAY 4, 1984

Federation Proceedings

Official Publication of the
FEDERATION OF AMERICAN SOCIETIES
FOR EXPERIMENTAL BIOLOGY

FUTURE FASEB AND SOCIETY MEETINGS

ASBC/AAI—JUNE 3-7, 1984, St. Louis, MO
APS—JULY 29-AUGUST 3, 1984, Lexington, KY
ASPET—AUGUST 19-23, 1984, Indianapolis, IN
ASCB—NOVEMBER 13-17, 1984, Kansas City, MO
FASEB—APRIL 21-26, 1985, Anaheim, CA

FEDERATION PROCEEDINGS is published 15 times a year, monthly except 2 issues in March and 3 in May, by FASEB. All issues except *March* (nos. 3 and 4) and *May 1* and 4 (nos. 6 and 7) contain papers presented at symposiums and articles especially written for FP. The *March* issues contain the abstracts of papers submitted for presentation at scientific sessions at the Annual Meeting of the Federation. The *May 1* and 4 issues (nos. 6 and 7) contain the abstracts of papers submitted for presentation at scientific sessions of the Annual Meeting of the American Society of Biological Chemists and The American Association of Immunologists. The views expressed in articles and in meeting abstracts are those of the authors and not necessarily those of the Federation. Manuscripts that are considered to be of interest to readers of FP are welcome. All manuscripts submitted (including FASEB or constituent Society symposiums as well as any others submitted) are subject to review and approval by the Editors before publication in FP. Rejected manuscripts may be reconsidered if appealed through the Editorial Board. Second-class postage paid at Bethesda, Maryland, and at additional mailing offices. Copyright © 1984 by the Federation of American Societies for Experimental Biology, 9650 Rockville Pike, Bethesda, Maryland 20814. ISSN 0014-9446. Printed at Lancaster Press, Lancaster, Pennsylvania. All rights reserved. Requests for any reproduction of copyrighted material except first page of a regular article should be made in writing to the Executive Editor at 9650 Rockville Pike, Bethesda, Maryland 20814, and should include an explicit statement of intended use and detailed specification of the material to be reproduced. Telephone 301/530-7119. COPYRIGHT: The code at the bottom of the first page of an article indicates the copyright owner's consent that copies of the article may be made provided that the stated fee is paid through the Copyright Clearance Center, Inc., 21 Congress Street, Salem, Massachusetts 01970.

Business Manager: All business matters should be directed to Mr. Austin H. Henry, Business Manager, FASEB, 9650 Rockville Pike, Bethesda, MD 20814 (301/530-7066).

Advertising Representative: From *Steven K. Herlitz, Inc.*, Steven K. Herlitz, Inc., 404 Park Avenue South, 9th Floor, New York, NY 10016 (212/532-9400).

Advertising Production Coordinator: Stella Koerber, in care of Steven K. Herlitz, Inc., 404 Park Avenue South, 9th Floor, New York, NY 10016 (212/532-9400).

Subscription Price: Members of Constituent Societies \$12.00 per year; Nonmembers (Personal) \$65.00 per year; Institutional \$85.00 per year; Students \$35.00 per year with certification. **Postage Differentials:** Other Countries + \$20.00. All subscriptions entered on a calendar year basis only and payable in advance. **Single Issues:** \$12.00, except March 1 and 5, Nos. 3 and 4 (*Abstracts*), \$35.00 and May 1 and 4, Nos. 6 and 7 (*Abstracts*), \$35.00. Subscriptions and orders should be sent to: FEDERATION PROCEEDINGS, Subscription Department, 9650 Rockville Pike, Bethesda, Maryland 20814 (301/530-7027).

Contents

ABSTRACTS

Continued from Issue Number 6, May 1, 1984

WEDNESDAY AFTERNOON June 6, 1984 (continued)

Blood coagulation I (continued)	1841
Exocytosis and secretion	1843
Ribosomes	1847
Nonmuscle contractile and cytoskeletal systems I	1849
Collagen, laminin and elastin: metabolism and molecular biology	1851
Proteolytic enzymes I	1853
Protein processing and turnover I	1856
Water-soluble vitamins	1859
Fat-soluble vitamins	1860
Fatty acid metabolism I	1863
Protein sequences II	1867
Enzyme-multienzyme complexes I: dehydrogenase complexes	1869
Enzyme kinetics I	1872
Bioenergetics I	1874
Oxygenases	1879
Protein kinase II	1881
DNA and RNA: conformation	1884
Oncogenes	1886
Mutagenesis	1887
Peptide growth factors I	1888
Peptide hormones: receptors and action	1890
Insulin	1892
Phosphoprotein phosphatases	1896
Steroids	1900

THURSDAY MORNING June 7, 1984

Transplantation immunity and biology	1904
--------------------------------------	------

Natural killer cells (nontumor targets)	1906
Cloned lymphoid cells with effector function	1908
Regulation of B cell function and isotype expression	1909
Immunoglobulin receptors, lymphocyte markers	1914
Disorders of immune regulation in humans	1918
Cellular and humoral mediators of inflammation	1924
Effect of lymphokines, antibodies and immunotherapeutic agents on tumors	1928
Basophils, mast cells and IgE	1933
Clinical disorders of immediate hypersensitivity	1935
Viruses and immunological abnormalities	1937
Biochemical pharmacology II	1940
Blood coagulation II	1944
Protein kinase III	1947
Peptide growth factors II	1950
Action of toxins	1952
Fibrinolysis	1957
Enzyme kinetics II	1958
Proteolytic enzymes and inhibitors	1961
Cell surface proteins and glycoproteins	1964

THURSDAY AFTERNOON June 7, 1984

Cellular interactions	1967
Cellular receptors for immunoglobulin	1969
Effect of lymphokines, antibodies and immunotherapeutic agents on tumors	1970

Basophils, mast cells and IgE	1972
Viruses and immunological abnormalities	1974
Membrane-bound enzymes in the regulation of blood clotting	1975
Translational control in eukaryotes	1976
The secretory pathway	1978
Cell surface recognition	1978
Gene structure and expression in plants	1979

WEDNESDAY AFTERNOON June 6, 1984

Peptide growth factors and their mechanisms of action	1979
---	------

THURSDAY AFTERNOON June 7, 1984

Transplantation immunity and biology	1980
NK cells and nonspecific effector cells	1986
Autoimmunity III	1991
Cell lines and clones with effector function	1995
Coenzymes	1998
Bioenergetics II	1999
Neurochemistry	2004
Enzymes: mechanisms of action III	2007
Enzyme-multienzyme complexes II	2012
Nonmuscle contractile and cytoskeletal systems II	2015
Protein synthesis: mechanism	2017
Protein processing and turnover II	2021
Phospholipids-lipids III	2025

Chromatin and nuclear proteins	2029	Endocytosis and receptor-mediated uptake	2043	Flavoproteins	20
Cytochrom P-450 III: structure and function	2032	DNA and RNA: chemistry ...	2047	Histones, chr matin-associated proteins and poly(ADP-ribose)	20
Glycoproteins III	2036	Eukaryotic gene expression III	2050	Proteolytic enzymes II	20
Fatty acid metabolism II	2040	Transport III	2053	Additional abstract	20
		Metalloproteins	2057		

INDEXES

Author index	2068
Permuted title index	2088

The abstracts on pages 1841-2067 were prepared by the authors and then printed by photo-offset without change. Abstracts are not subject to scientific review; therefore, the scientific validity of the results reported is the responsibility of the authors and sponsors. Accuracy, form of citation, designation of materials, acknowledgment of all co-authors and of grant support, terminology, nomenclature, and the like, all remain again the responsibility of the authors and sponsors. Readers should note that the appearance of an abstract in this issue does not necessarily imply future publication of a regular scientific paper.

Asterisk (*) indicates nonmember of Society to which an abstract was submitted.

It is our pleasure to record the help of the staff of BIOSIS in preparing the indexes, particularly from Ms. Carolyn Marconi and Mr. John R. Thomas.